VOLUME XXVIII NUMBER 4

BOTANICAL GAZETTE

OCTOBER 1899

THE COMPOUND OOSPHERE OF ALBUGO BLITI.

CONTRIBUTION FROM THE HULL BOTANICAL LABORATORY XVI.

F. L. STEVENS.

[*Concluded from p. 176.*]

RIPENING OF THE OOSPORE.

THE process by which the oospore attains its mature condition is easily divided into three stages, which are marked by changes that take place in the oospore wall, as follows: (1) a period during which the exospore is partially constructed, and which terminates with the beginning of (2) when the primary endospore is laid down and the exospore nearly completed, and (3) a period during which the secondary endospore is formed and the oospore becomes fully ripe and ready for its winter rest.

The nuclei in the oosphere withdraw from the immediate vicinity of the primitive wall soon after fusion of the sex nuclei, or even before this act is completed, and the strands of cytoplasm toward the center thicken, leaving those of the periplasm but little more than slender supporting threads. This condition is difficult to describe but is well represented in *fig. 89*. The vacuoles have previously enlarged and the cytoplasm is more granular than at any earlier stage. The primitive wall becomes about 0 75 μ thick.

At this time a peculiar substance appears in the interstices of the ooplasm and fills the large vacuoles. It is not seen in younger stages, but is constant at this period and takes a rusty color when Flemming's triple stain is used. When the substance first appears the tint is barely perceptible, but later it assumes a brick-red color. Slightly stained sections show a film in the vacuoles, often so curled up that an edge view is obtained, thus showing that the vacuoles actually contain a stainable substance. This substance is present in stages such as those represented in *figs. 91, 92, 93, 94*.

As the primitive wall reaches maturity it becomes covered by a layer of semi-transparent substance which is penetrated by straight pores. The condition is illustrated in *fig. 92*, where a portion of this layer lies in such a position that both a surface and an edge view appear at one time. As the pores are close together and straight the structure reminds one of the hymenial surface of the Polyporei. Since the main substance of the layer is nearly invisible, the pores appear dark and look like papillæ or cilia growing out of the primitive wall when seen in edge-view (*fig. 92*). A condition was noted by Wager in *A. candidus*, and referred to as the "columnar condition," which from the descriptions and drawings seems similar to this. There appear somewhat later in this semi-transparent layer peculiar saucer-shaped masses of a dark substance of unknown nature (*fig. 91*). They are the first indication of the ridges so characteristic of the mature epispore. There is but little periplasm at this period, although the nuclei of the periplasm are apparently still undiminished in number. However, they stain very lightly and show only a membrane and nucleolus. They frequently mass together in bunches, but there is no evidence that they disorganize to furnish material for the exospore as is described for *A. candidus*.

To complete the endospore the saucer-shaped regions, previously mentioned (*fig. 91*), extend laterally and run together, thus forming the ridges. This gives the epispore the structure shown in *fig. 93*. Nuclei persist much longer outside of the exospore, but they seem to be functionless, and their history was not followed

further. The mature endospore consists of two layers that may be called the primary and secondary endospores according to their development. The primary endospore appears as an even layer lining the primitive wall, at first agreeing precisely in stain reaction with the gelatinous material of the vacuoles, which is still present (*fig.* 93). The primary endospore, however, soon refuses this stain and gives the reaction of cellulose with chlor-iodide of zinc. A variation in the normal sequence was found in one oospore in that the epispore was completed before any trace of endospore was to be seen.

The conspicuous feature of the third period is the laying down of the secondary endospore inside of the first. It is of cellulose, and is about equal to the primary endospore in thickness. The two layers of the fully developed endospore are clearly evident when stained, also when they cleave apart in a perfectly regular manner as they frequently do (*fig.* 97). There seems to be a pause in the laying down of cellulose when the primary endospore is completed, as is shown by the fact that perceptible changes occur in the development of the epispore after the first and before the second endospore is formed. *Fig.* 94 shows the earliest trace of the secondary endospore that was seen. When the second layer of cellulose is completed the gelatinous material has completely disappeared from the vacuoles. The fact that this substance appears just before the laying down of the primary endospore, the agreement in their staining reactions while the endospore is young, and the disappearance of all of the gelatinous material simultaneously with the completion of the secondary endospore, are significant facts. They indicate that this substance is intimately connected with the formation of the cellulose walls of the spore. It appears to be transferred directly from the vacuoles to the exterior of the protoplasm, there to change to true cellulose.

Finally, various food substances accumulate in the center of the oospore, taking the form of one very large irregularly shaped globule (*fig.* 97). Around this mass lies a zone of cytoplasm containing the fusion-nuclei in resting condition. Their number

is about one hundred, so it is not probable that there has been any multiplication since fertilization. Mitotic figures have never been seen in the oospore after that act, and it is probable that the oospore persists in this condition until the following spring.

OILS AND OTHER FOODS.

A thorough discussion of this subject would involve a much more elaborate microchemical study than was undertaken. It seems desirable, however, to record a few observations since these extra-protoplasmic substances are very conspicuous at certain periods of development and their presence often introduces serious difficulties of technique. No writer has given the subject the attention that it deserves, and detailed study would doubtless yield valuable results.

There are three substances which, when present, always appear as nearly spherical globules with the general appearance of oil, and they have undoubtedly been described as such by many previous observers. Further study may show that they are not true oils, and this seems quite probable, as they do not answer to all of the microchemical tests; but in the lack of a precise knowledge of their chemical nature they may be considered here under that name. All of them are found in the serial sections cut from paraffin, that is, in material which has passed in bulk through the weak alcohols and rested in 75 per cent. alcohol for weeks or months; which has passed to the paraffin through chloroform and been cleared of paraffin by immersion for a few moments in xylol; and finally which has undergone the baths accessory to the stain employed.

The first oil is very abundant, existing in far greater quantity than either of the others, and during more stages in the development of the fungus. It is found in the young oogonium, the oosphere, and the periplasm. Diagrams showing its distribution in the important periods of development are given in the plates. *Fig. 46* presents it for an oogonium of about the age shown in *fig. 45*. *Fig. 63* illustrates its distribution at zonation, and it should be noticed that the drops are small where the

meshes of the ooplasm are fine, but large in the periplasm where the meshes are more coarse. *Fig. 72* shows the condition of an oogonium at a stage similar to *fig. 70*, a period somewhat later than zonation. The distribution is much the same because the protoplasm has changed but little. *Fig. 81*, a stage after fertilization, indicates that as the meshes of the ooplasm become coarser the globules become larger; apparently some of the globules fuse to form the larger drops. A later stage similar to *fig. 93* shows that the oil of the periplasm is nearly exhausted during the building up of the epispore.

This most common oil is found in unstained sections as blackish or brown drops when the material has been killed by Flemming's fixing fluid. It is usually absent from material fixed by chrom-acetic acid, or if present is in very small quantity. If the material is allowed to remain several (six) days in the chrom-acetic fixing agent the oil appears in considerable quantity, very much as it does in Flemming's material, but of lighter straw color instead of brown. When material fails to show the oil, it seems to be really absent, because several days' immersion in 1 per cent. osmic acid solution fails to show its presence. Therefore, it seems that the osmic acid of the Flemming's fixing fluid, or the long immersion in chrom-acetic acid, so acts on the oil as to render it less easily soluble in the fluids that it meets between the killing and the time when the sections are stained. This oil when present takes a characteristic and beautiful red color from the safranin in the Flemming's triple stain combination. If it be very dark from the action of the osmic acid, it is frequently necessary to employ hydrogen peroxid to remove the blackness, in order that the best results may be obtained from the stains. It is partially on account of this oil that material killed by Flemming's fluid is not so favorable for cytological study as that fixed by the chrom-acetic acid.

The second oil-like substance is present only in very small quantity, possibly eight or ten small globules in an oogonium. It is evident in both Flemming and chrom-acetic material, is

stained black by Heidenhain's hæmatoxylin, but may be distin guished from the first oil by the fact that it does not take the safranin when applied in the usual way. It is figured in the plates as black dots (*figs. 51, 56, 59, 61, 64, 68, 69*); in the antheridium in *fig. 85;* in the periplasm in *fig. 80.* No difference in stain reaction was observed between this oil-like substance and the central globule of the cœnocentrum, and they may be of similar nature.

The third oil is only found in the maturing oospore, first appearing while the secondary endospore wall is developing as globules of a clear honey-yellow color in the meshes of the protoplasm (*fig. 95*). It soon accumulates at the wall, and *fig. 94* shows large drops in close contact with the forming secondary endospore, which has broken away from the primary endospore, probably owing to the impact of the knife in cutting. The drops finally become larger and more numerous, as is shown in *fig. 96.* From a study of subsequent stages it seems probable that these oil drops later break away from the wall irregularly, and unite to form the several large drops that are often found in older oospores. This results finally in the condition shown in *fig. 97,* where the entire central region is occupied by a curious irregular globular structure which stains much as the oils have stained in previous stages, but which is certainly not of fluid consistency. It probably represents reserve food material. If this structure represents a genuine oil drop in the living spore, we may have to do here with the shrunken and collapsed vesicle or membrane that encased it in life, and whose contents have been lost during the processes of technique. Its appearance would accord well with this view. From its size and the time of development in the oospore it is surely the structure described as oil by previous investigators, and is characteristic of the winter oospore.

GENERAL CONSIDERATIONS.

It seems well before closing to take a general view of the facts that have been presented, and of their relation to previous knowledge; also to point out more clearly than was possible

in earlier pages the broader significance of the phenomena described.

A mitotic division of the nuclei of fungi seems to have been first noticed by Sadeback ('83) in Exoascus, and has since been observed in various forms and carefully studied and described in a few papers.

The occurrence of a mitotic division in the oogonium of Albugo was discovered by Wager ('96) in *A. candidus*, and confirmed by Berlese ('98) in *A. Portulacæ*. Wager notes that the nuclei enlarge and divide, leaving one daughter nucleus imbedded in the central body while the other nuclei pass to the periplasm. The one division increases the nuclei from about 115 to about double that number. Berlese says the nuclei divide several times in *A. Portulacæ*, increasing the number from 30 or 40 to about 200. The account given in the present paper describes two approximately simultaneous divisions affecting all of the nuclei in both antheridium and oogonium, and these mitoses result in the formation of the sexual elements, numerous male and female nuclei. The second division is strikingly different from the first in the appearance of the nuclear elements, particularly the chromatin. This condition suggested the possibility of a reduction of chromatin, but careful study revealed no convincing evidence. The mitoses are characterized by the intranuclear formation of the spindle, the intranuclear centrosomes, the permanence of the nucleolus, and the entire absence of extranuclear radiation.

Wager described the disappearance of the nucleolus in early prophase, followed by the formation of chromosomes and then by the spindle development. He inclines toward the view that the spindle is derived from the linin. The membrane persists up to metaphase, and Wager did not follow the division further. Berlese, from observations similar to those of Wager, namely, the disappearance of the nucleolus in prophase, argues that the spindle fibers are derived from it. The behavior of the nucleolus in *A. Bliti* is different from that described by the writers mentioned, in that the structure remains apparent inside of the

nucleus through all stages of mitosis. However, since Wager studied this question only incidentally, and as Berlese gives no figures and his account is very brief, a detailed comparison of the species is impossible. Berlese reports from twelve to sixteen chromosomes, and seems to have been able to count them during the fusion of the sexual nuclei. Neither Berlese nor Wager give details of the mitosis later than prophase.

Wager reports that as near as he can estimate there are from twelve to sixteen chromosomes shown in the mitotic figures. In *A. Bliti* six are found with certainty in some anaphase nuclei, and twelve appear in some metaphase nuclei with equal certainty. It may be that when twelve are counted the chromosomes have already divided, and that they really belong to two rather than to one nucleus. However, this is not certain, and there is some evidence that makes it appear that there is a reduction in the number of chromosomes during the first mitosis, but this cannot be considered as proved.

Centrosomes were not observed in either *A. candidus* or *A. Portulacæ*, or in any other of the Phycomycetes, so far as the writer is aware; but they have been described in earlier papers for Ascomycetes (Gjurasin '93, Harper '95), and for Basidomycetes (Wager '92, Juel '98).

With such fundamental differences as have been indicated, it is useless to attempt to establish a type of mitosis for Albugo, or to attempt to determine the relationship of the group through cytology. The type here described for *A. Bliti*, while presenting a few deviations from the mode of mitosis in vogue among higher plants, is in no way a departure from the forms well known among the lower types of plants and animals.

It is impossible to generalize on the facts of morphology presented in this paper, because simple processes of fertilization have been described in all forms where homology would be sought. Until the behavior of the nuclei of other species of Albugo is known, it is impossible to say which is the anomalous form of the genus, *A. candidus* or *A. Bliti*. From a preliminary study of *A. candidus* it can be affirmed that *A. Bliti* is in most

ways far more favorable for study; its nuclei are larger, its periplasm more abundant, its developmental stages more strongly marked, and its antheridial tube larger. *Albugo candidus*, however, has a remarkable cœnocentrum, which will be much easier to study than that of *A. Bliti*, owing to its much greater size and more pronounced stain reaction. A problem of great importance lies in a comparative study of the cœnocentra of the genus.

The characteristic massing of the cytoplasm to form a rudimentary oosphere in *A. Bliti*, differing thus from the vacuolate oosphere of *A. candidus*, is not a wider divergence than might be expected in different species; nor is the variation in the cœnocentrum more than what might be regarded as a specific difference. If such variations are found to be more marked in other species the way may be clear to trace the relationship between plants with one oosphere and those with several oospheres in each oogonium; between forms which differentiate their periplasm after the manner of Vaucheria, and others that follow the habit of the Saprolegniaceæ in a parietal rather than a central massing. It must be left to future research to make clear the relationship that must exist between the multiple fertilization illustrated by *Albugo Bliti* and simple acts of fusion between sexual elements.

It may be that cytological investigation will show remarkable variations in many respects in this genus, and establish a chain of derivative forms. The Saprolegniaceæ are said to range from parthenogenesis to complete sexual fertilization. Should Albugo prove to be similarly rich in habits the present knowledge of relationships will be much increased.

METHODS, MATERIALS, AND STAINING REACTIONS.

The material upon which this investigation was based consisted of leaves, stems, and flower clusters of *Amaranthus retroflexus* L. and *A. hybridus* L. bearing the fungus. It was collected at Syracuse, N. Y., Columbus, Ohio, and Chicago, Ill. In all cases the species seemed to be unquestionably *Albugo Bliti* Biv.

(*C. Amaranti* Schw. *C. amarantacearum* Zal.). The form described by Zalewski ('83) as a different species was not met. Oospores are very abundant on both leaves and stems, producing on the former characteristic blister-like patches that assume a blackish hue if the oospores ripen in sufficient quantity. In the stems their presence may be predicted from peculiar swellings, usually accompanied by a reddish coloration, the entire plant often being thus affected. In partially diseased plants the oospores are likely to be found in the inflorescence, which reacts much as does the stem, becoming swollen and red. The parts most favorable for study are the stems, but leaves and flowers often section more easily. In killing the material undesirable parts were cut away, and the portions apparently favorable were cut into small bits; leaves were scored, and stems and peduncles were cut in pieces about 2 to 4^{cm} long, deep incisions being made every 2^{mm} to give ready access to the killing agent. The killing with suitable solutions was apparently perfect, and was as good midway between incisions as where the solution immediately reached the tissue.

The killing agent giving the best results was chrom-acetic acid of the following formula: chromic acid 0.8 per cent., acetic acid 0.5 per cent. in water. The material was usually left in this solution from twelve to eighteen hours, then washed in five or six changes of water, allowing about two hours between changes. It was then successively transferred to 12, 25, 50, 75 per cent. alcohol, remaining about two hours in each grade, and was left in the last grade until it was practicable to imbed in paraffin. A variation in the above method, by which the material was left six days in chrom-acetic acid gave interesting results. It rendered the oil in the protoplasm much less soluble, but caused the loss of many of the details of the mitoses.

Flemming's chrom-osmic-acetic acid was employed in the same manner as the chrom-acetic acid, but was not so useful, since the sections were very much darkened by the osmic acid, thus necessitating elaborate methods of bleaching before a desirable stain could be obtained.

Other killing agents used were corrosive sublimate in saturated aqueous solution; hot corrosive-acetic-sublimate in alcohol; Carnoy's fluid; absolute alcohol; Hermann's fluid; and Merkel's fluid. Most of these gave far inferior results to that obtained by the chrom-acetic acid, and none surpassed it in effect.

In order to imbed in paraffin the material was transferred through 85, 95, and 100 per cent. alcohol to a mixture of absolute alcohol and chloroform, first of one third then of two thirds strength of chloroform. The specimens were left about two hours in each fluid, and were finally placed in pure chloroform. After the material had been left in chloroform for an hour a quantity of paraffin was added, and two hours later the material was warmed on the bath. After two hours of gentle heat it was removed to a warmer position, and later the most of the chloroform was poured off, melted paraffin substituted, and the whole kept in the bath at a temperature of about 55°. It was found advantageous during the whole process of imbedding to use very shallow dishes, as by this means most or all of the chloroform is driven off gradually by evaporation. Material was left in the bath in soft paraffin three or four hours, the paraffin being twice changed in the meantime to insure the removal of all chloroform. It was then cast in a cake, the final paraffin having a melting point of about 62°. Sections 3 to 5 μ in thickness were cut with a Jung sliding microtome and fastened to the slide with Mayer's albumen fixative.

Flemming's triple stain, used with chrom-acetic acid material, gave the best results. This stain demands the greatest attention in its use or failure is inevitable, as is well known by all who employ it. In general, the best results were attained by a bath of 30–60 min. in safranin, followed by a rinsing more or less prolonged in acid alcohol. The time here is entirely a matter of judgment, varying with the result desired; 30–90 sec. was a most usual time. After running down through the alcohols the slide was placed in saturated solution of gentian-violet for from 5–45 min. It was then rinsed in water and placed in orange

G from 5–25 sec.; a longer time may do no harm but probably 10–15 sec. is always sufficient. Wipe away excess of liquid and flood the slide twice with absolute alcohol, allowing the second lot of alcohol to remain on the slide until sufficient of the gentian-violet has been removed. The time required will depend on the material, the length of time it was in the gentian-violet, and the result desired. Drain rapidly with filter paper and flood with clove oil for one minute; drain, follow by cedar oil and cover in xylol balsam. If properly stained the host cell wall should be a light violet, the chromatin of the spirem and the chromosomes blue, nucleolus and centrosomes red, and cytoplasm slightly yellowish.

Hæmatoxylin stain was used, applied after the method of Heidenhain (iron-alum 2 hrs., hæmatoxylin 12–18 hrs., followed by the slow extraction of iron alum till the proper degree is reached). This treatment gave some beautiful results in contrast with the Flemming stain, and was particularly valuable in demonstrating the achromatic portion of the nuclear figure. Hartog's nigrosin-carmine stain, as used by Wager and Berlese, was tried repeatedly on corrosive sublimate material, but the results were far inferior to those afforded by Flemming's triple or Heidenhain's hæmatoxylin stains. However, it is possible to demonstrate, even with this combination, the presence of many nuclei in the oosphere and in the antheridial tube, and to recognize the principal features of the mitotic figures. Other stains employed were Delafeld's hæmatoxylin, Biondi-Ehrlich, and cyanin-erythrosin, but they were distinctly inferior in their results.

The following stain reactions were presented in the best preparations, and were attained by the Flemming's triple stain unless otherwise stated: chromatin blue or violet, black with Heidenhain's hæmatoxylin; nucleoli red, black with Heidenhain's hæmatoxylin; centrosomes as nucleoli; spindle fibers dark blue; cytoplasm yellowish; granules, mentioned on page 163, are only seen in preparations stained with Heidenhain's hæmatoxylin, and then black.

SUMMARY.

1. The oogonium when cut off from the parent hypha contains about 300 nuclei, which enlarge and divide mitotically while the oosphere is being differentiated.

2. The oosphere is differentiated through a massing of the cytoplasm of the oogonium. By this process the nuclei, usually in stages of mitosis, together with the vacuoles, are expelled from the central region, and there results a dense and coarsely vacuolate periplasm. This condition occurs when the antheridial tube is very short.

3. There is a stage called zonation in which the nuclei, usually in metaphase, are lined up around the ooplasm, some of the spindles lying across the definite boundary that separates ooplasm from periplasm. In the telophase of this mitosis about fifty daughter nuclei are found in the ooplasm.

4. The antheridium contains at first about thirty-five nuclei which divide twice mitotically, and simultaneously with the division in the oogonium and oosphere.

5. Previous to the entrance of the antheridial tube a papilla is found projecting from the oogonium into the antheridium.

6. The antheridial tube penetrates slowly, reaching the ooplasm at the time of zonation, later entering the oosphere and appearing as a conspicuously multinucleate structure. When it opens there are discharged about one hundred male nuclei which fuse with the female nuclei in pairs.

7. The sexual nuclei differ in form; the sperm being elongated and the egg spherical.

8. A peculiar central body, the cœnocentrum, develops as the oosphere matures and disappears before fertilization. Its function is unknown. There is some evidence of its being a dynamic center of the compound oosphere.

9. The mitoses are alike in the oogonium and antheridium. The spindle is intra-nuclear and there are no extra-nuclear radiations. The centrosomes are very prominent at metaphase, and are intra-nuclear. They could not be distinguished in the

resting nucleus. The nuclear membrane persists until after metaphase and the nucleolus is present throughout the division.

10. The primitive wall of the oospore first appears when the antheridial tube opens. Later the epispore is laid down upon it by the periplasm.

11. Two endospores are formed by the ooplasm after the development in the vacuoles of a peculiar substance which disappears as the endospores reach maturity.

12. After the complete encasement of the oospore it becomes rapidly filled with food-stuffs. A large central oil-like drop is present during the winter condition,

13. The fusion nuclei pass the winter in the resting condition without further perceptible change.

THE UNIVERSITY OF CHICAGO.

BIBLIOGRAPHY.

BEHRENS, 90. Einige Beobachtungen über die Entwickelung des Oogons und der Oosphäre von Vaucheria. Ber. d. deut. bot. Gesells. **8**: 314.

BERLESE, 98. Ueber die Befruchtung und Entwickelung der Oosphäre bei den Peronosporen. Jahrb. f. wiss. Bot. **31**: 159.

DANGEARD, 90. Recherches histologiques sur les champignons. Le Bot. **1890**: 125.

GJURASIN, 93. Ueber die Kernteilung in den Schläuchen von *Peziza vesiculosa* Bull. Ber. d. deut. bot. Gesells. **11**: 113.

HARTOG, 95. On the cytology of the vegetative and reproductive organs of the Saprolegniaceæ. Trans. Roy. Irish Acad. **30**: 645.

HUMPHREY, 92. The Saprolegniaceæ of the United States with notes on other species. Trans. Am. Phil. Soc. **17**: 63–148.

HARPER, 95. Beiträg zur Kenntniss der Kernteilung und Sporenbildung im Ascus. Ber. d. deut. bot. Gesells. **13**: 67.

ISTVÁNFFI, 95. Ueber die Rolle der Zellkerns bei der Entwickelung der Pilze. Ber. d. deut. bot. Gesells. **13**: 456.

JUEL, 98. Die Kernteilung in den Basidien und die Phylogenie der Basidiomyceten. Jahr. f. wiss. bot. **32**: 361.

LÉGER, 95. Structure et developement de la zygospore du *Sporidinia grandis*. Rev. gén. Bot. **7**: 481.

OLTMANNS, 95. Ueber die Entwickelung der sexualorgane bei Vaucheria. Flora 80: 388.

SADEBECK, 83. Untersuchungen über die Pilzgattung Exoascus. Jahrb. der wiss. Anstalten zu Hamburg —: 101.

TROW, 95. The karyology of Saprolegniaceæ. Ann. Bot. 9: 609.

——— 95. Trans. Roy. Irish Acad. 30: 649.

WAGER, 90. Observations on the structure of the nuclei in *Peronospora parasitica*, and on their behavior during the formation of the oospore. Ann. Bot. 4: 127.

——— 93. On the nuclei in Hymenomycetes. Ann. Bot. 6: 146.

——— 96. On the structure and reproduction of *Cystopus candidus* Lév. Ann. Bot 10; 295.

ZALEWSKI, 83 Zur Kenntniss der Gattung Cystopus. Bot. Cent. 15: 215.

ZIMMERMANN, 96. Physiologie des pflanzlichen Zellkernes 129, 146.

EXPLANATION OF PLATES XI–XV.

All figures are from material killed in chrom-acetic acid and stained with Flemming's triple stain, unless otherwise indicated. The figures were sketched with an Abbé camera, using the following combinations of lenses: Zeiss 2^{mm} ap. 1.30, with compensating ocular 18 and 12; also Bausch & Lomb $\frac{1}{12}$, with ocular no. 4. These combinations give respectively the following magnifications when projected to the table level: 3300, 2200, and 1500 diameters. *Plate XI* was not reduced in reproduction. All other plates are three fourths of the original scale.

PLATE XI.

All drawings represent a magnification of 3300 diameters, and were studied with Zeiss lenses.

FIG. 1. Resting nucleus in mycelium, linin faint, nucleolus prominent.

FIG. 2. Nucleus in flowing cytoplasm near entrance of oogonium.

FIG. 3. Nucleus in antheridium, spirem stage, membrane faint.

FIG. 4. Prophase: small drops accumulated on the linin network, the thread itself thinner, nucleus becoming spindle-shaped, no centrosome visible.

FIG. 5. A stage somewhat later, linin threads have almost entirely disappeared, chromatin grains scattered through nucleus, nucleolus to the right, and remains of a linin strand to the left.

FIG. 6. Nucleus more spindle-shaped, globules arranged in the equatorial plate, at the poles round bodies about equal to the chromatin dots in size, no spindle fibers, the definite line bounding the whole of the nuclear membrane.

FIG. 7. Similar to *fig. 5*. The longitudinal lines probably chromatin which has not reached the equator, nucleolus at the left. This nucleus was crowded by a mass of others, hence its short form.

FIG 8. Similar to *fig. 7*. Fibers visible toward the poles. This spindle was formed in one of the strands supporting the forming oogonium, and was consequently much elongated by tension. It is from the oogonium shown in *fig. 62*.

FIG. 9. Chromosomes at the equator, spindle fibers very apparent at the poles, but not visible at the equator, nucleolus to left, membrane intact and inclosing the spindle.

FIG. 10. From oogonium shown in *fig. 60*. Cross section of a spindle, twelve chromosomes apparent (stained by hæmatoxylin from Flemming's material).

FIG. 11. Spindle mature, chromosomes closely grouped at the equator, centrosomes prominent, spindle brilliant and clear, nuclear membrane present but poorly stained (*fig 13*).

FIG. 12. Chromosomes splitting, membrane visible with nucleolus enclosed.

FIG. 13. Nucleolus large, nuclear membrane very definite, daughter chromosomes ready to separate. Only those in the highest focus are shown, several others being found at a deeper focus. The stain was particularly to show the membrane, and was not suitable for centrosomes.

FIG. 14. Anaphase: chromosomes separating, nucleus lying about midway, centrosomes still visible, whole nucleus staining dark and membrane indistinguishable from spindle fibers.

FIG. 15. Chromosomes nearing the poles, centrosomes not distinguished from them, nucleolus midway, slight traces of spindle fibers stretching across the middle space, cytoplasm in the ends of the nucleus stains darker than that of the central area.

FIG. 16. Whole spindle elongated, chromosomes massing together at the poles.

FIG. 17. Similar to *fig, 16*, but in a crowded position. Compare with *fig. 7*.

FIGS. 18 and 19 are from the periplasm of an oogonium of about the age shown in *fig. 67*, slightly younger than shown in *fig. 68*.

FIG. 18. Spindle fibers collapsing in the middle leading to the separation of the daughter nuclei, the fibers constituting the origin of the membrane of the daughter nucleus.

FIG. 19. A young daughter nucleus, nucleolus, membrane, and chromosomes.

FIG. 20. Same as *fig. 19* in resting stage, nucleolus prominent, linin faint.

FIGS. 21 and 22 are from the same oosphere, and in the condition shown in *figs. 68-70.*

FIG. 21. Same as *fig. 20* but passing into the spirem stage.

FIG. 22. Nucleus elongating preparatory to division. Compare *fig. 4.*

FIG. 23. Breaking of skein into chromosomes, centrosomes apparent.

FIG. 24. Spindle forming inside of nuclear membrane, nucleolus lying outside of the spindle.

FIG. 25. From an oospore of the condition shown in *fig. 70.* Spindle lying completely inside of the nuclear membrane, chromosomes grouping at the equator, and centrosomes well defined.

FIG. 26. Metaphase: chromosomes splitting, membrane, centrosomes, and fibers clear. Fibers apparently of about the same number as the chromosomes.

FIG. 27. Daughter chromosomes ready to leave the equator.

FIG. 28. Anaphase: chromosomes well separated, centrosomes visible, spindle fibers crossing the middle space, chromosomes six in number. From same oospore as *figs 24* and *27.* Compare with *fig. 16.*

FIG. 29. Later anaphase: chromosomes near the poles, area in which they rest darker stained than central portion.

FIG. 30. Similar to *fig. 18.* Spindle fibers collapsed and daughter nuclei ready to separate. From same oospore as *fig. 29.*

FIG. 31. A male nucleus from the entrance of antheridial tube. This nucleus is the same as the one marked X in *fig. 73.*

FIG. 32. A nucleus (sperm) in the tip of the same tube that contained that shown in *fig. 31,* also marked X. Wall of the antheridial tube, dense cytoplasm surrounding nuclei, sperm with nucleolus and mass of chromatin at anterior end.

FIG. 33. Tube open, elongated and pointed sperms escaping, showing a very faint linin network, one female nucleus shown. This is part of the drawing shown in *fig. 85.*

FIG. 34. A sperm approaching a female nucleus, linin more prominent than in *fig. 33.*

FIG. 35. A sperm in contact with a female nucleus, becoming more nearly round, and its linin still more prominent.

FIGS. 36 to 40. Various stages of fusion, from oospores of the general appearance represented in *fig. 90.* During fusion the whole nucleus becomes more darkly stained with gentian-violet.

FIG. 41. Coenocentrum with globule at its center, female nucleus near by, ordinary vacuoles of the oosphere near the margin.

PLATE XII.

Magnification in all figures 1500 diameters.

FIG. 42. A portion of mycelium, showing nuclei with prominent nucleoli.

FIG. 43. Nuclei and cytoplasm flowing into a developing oogonium, nuclei and vacuoles elongated and angular, nuclei too darkly stained to show structure.

FIG. 44. The septum below the oogonium, a portion of mycelium and posterior end of oogonium, oogonial nuclei assuming the spirem stage, mycelial nuclei still in resting condition with prominent nucleoli but enlarged.

FIG. 45. Oogonium and antheridium, nuclei of both in the spirem stage.

FIG. 46. Shows location of oil drops in an oogonium of the condition presented in *fig. 45*. They were all drawn at one focus; a slight change of focus would have brought vastly more into view.

FIG. 47. Showing adhesion of the oogonial *Hautschicht* to its wall in the neighborhood of the antheridium, nuclei overstained.

FIG. 48. Adhesion as in *fig. 47*. Oogonial wall partly and irregularly corroded away on the side toward the oogonium.

FIGS. 49–55. Stages in the perforation of the wall preparatory to the entrance of the antheridial tube.

FIG. 49. Optical section of papilla, wall partly corroded away and bulging toward the antheridium. The dense protoplasm represented in black was stained a deep red by the safranin.

FIG. 50. Sectional view of a condition slightly older than *fig. 49*. The shaded portion of the separating wall took the stain differently from the rest, and was apparently in the last stages of dissolution.

FIG. 51. A papilla of different shape in a stage similar to the last.

FIG. 52. Optical section, stain as in *fig. 49*.

FIG. 53. A papilla becoming bubble-like, walls very thin, slightly shrunken as though due to imperfect killing, watery vacuoles apparent, their contents staining homogeneously with the gentian violet.

FIG. 54. Bubble-like papilla expanding irregularly in all directions, contents highly vacuolate, wall extremely thin.

FIG. 55. Wall almost perforated, but showing no marked bulging in either direction, *o*, oogonial, *a*, antheridial side.

FIGS. 56, 57. Young antheridial tubes with a cellulose wall and surrounded by a dense sheath of the protoplasm of the oogonium.

FIG. 58. Protoplasm beginning to collect in masses, nuclei approaching metaphase, some shown in polar view (preparation from material fixed in Flemming's agent and stained by hæmatoxylin).

FIG. 59. An intercalary oogonium, slightly older condition than *fig. 58*, cytoplasm distinctly in masses, nuclei in early prophase, antheridial tube just entering bearing no nuclei, a haustorium at extreme right.

PLATE XIII.

Magnification in *figs. 60, 61, 62, 63, 64, 65, 68, 69, 70, 72,* 1500 diameters; in *figs. 66, 67, 71,* 3300 diameters; in *fig. 73,* 2200 diameters.

FIG. 60. Nuclei in metaphase, protoplasm massed in a few centers, spindles very clear and brilliant (stain hematoxylin from material fixed in Flemming's agent).

FIG. 61. Nuclei in late prophase, the protoplasmic masses coalesced to form one, vacuoles mark the juncture last made, a few nuclei not yet floated out.

FIG. 62. Nuclei approaching metaphase, spindles much elongated, all not yet extruded from the central region, mitosis in the antheridium, antheridial tube shown at its typical position for this stage.

FIG. 63. Diagram of disposition of oil drops in an oogonium of the condition shown in *fig. 65* (zonation).

FIG. 64. Nuclei nearly at metaphase, zonation almost complete, oil-like drop to the right in ooplasm, antheridial tube present showing no nuclei.

FIG. 65. Zonation: nuclei near metaphase, ooplasm sharply differentiated, many spindles lying directly across the boundary between ooplasm and periplasm (stained by hæmatoxylin from Flemming material). See also *fig. 64*.

FIG. 66. Shows spindle in metaphase lying across the film between ooplasm and periplasm.

FIG. 67. Nucleus in anaphase directly across the boundary film of the ooplasm, also half of a late anaphase cut diagonally.

FIGS. 68, 69. Consecutive sections of an oogonium just after the division of the nuclei, showing the position and shape of the antheridial tube. *Fig. 69* shows the cœnocentrum which had just passed its maximum development (see *fig. 71*).

FIG. 70. Antheridial tube and differentiating oosphere, oosphere nuclei in mitosis. *Fig. 74* was taken from an adjacent section of the same oogonium and shows the cœnocentrum.

FIG. 71. Completely developed cœnocentrum, the central globule surrounded by three regions of differentiated protoplasm. From a stage of zonation where the nuclei were in anaphase (as in *fig. 67*) and the daughter nuclei were about to pass into the ooplasm.

FIG. 72. Diagram of distribution of oil at the stage shown in *fig. 70*.

FIG. 73. Antheridium and tube of the age shown in *fig. 70*, showing multinucleate contents; *l*, film; ♀, one of the female nuclei; *p*, periplasm; *x, x*, nuclei, represented also in *Plate XI, figs. 31, 32*.

PLATE XIV.

Magnification in *figs. 74, 80, 82, 87, 88,* 1500 diameters; *fig. 76,* 2200 diameters; *figs. 75, 77, 78, 84, 85, 86,* 3300 diameters.

FIG. 74. Cœnocentrum and differentiated oosphere with dividing nuclei. The broken empty portion is where the protoplasm shrunk away from the antheridial tube. Section adjacent to that shown in *fig. 70.*

FIG. 75. Cœnocentrum, in an oogonium, at anaphase period of zonation, consisting of three small globules evidently fusing, surrounded by a region of denser protoplasm, granules resembling small oil drops scattered through the ooplasm.

FIG. 76. Similar to *fig. 73* but somewhat older, antheridial tube showing many nuclei, slightly torn near tip, antheridium becoming vacuolate; *p*, periplasm; *b*, film; ♀, female nucleus.

FIG. 76 *a*. Portion of the tip of the same tube, found in an adjacent section.

FIG. 77. View of the end of an unopened antheridial tube, wall not apparent, sperms very numerous and crowded, each showing a dark nucleolus, ooplasm slightly shrunken away from the tube.

FIG. 78. Section near the base of the same antheridial tube, showing very thick wall in contrast with the extremely thin film covering the apex.

FIG. 79. Diagram to show position of preceding sections. *Fig. 77* was from a section just above the line *aa*; *fig. 78* from one just below the line *bb*. One intermediate section was not drawn.

FIGS. 80, 80*a*. Adjacent and approximately longitudinal sections of the opening antheridial tube of the same oogonium, showing the primitive wall and the increased vacuolation of the ooplasm which frequently shrinks away from the primitive wall.

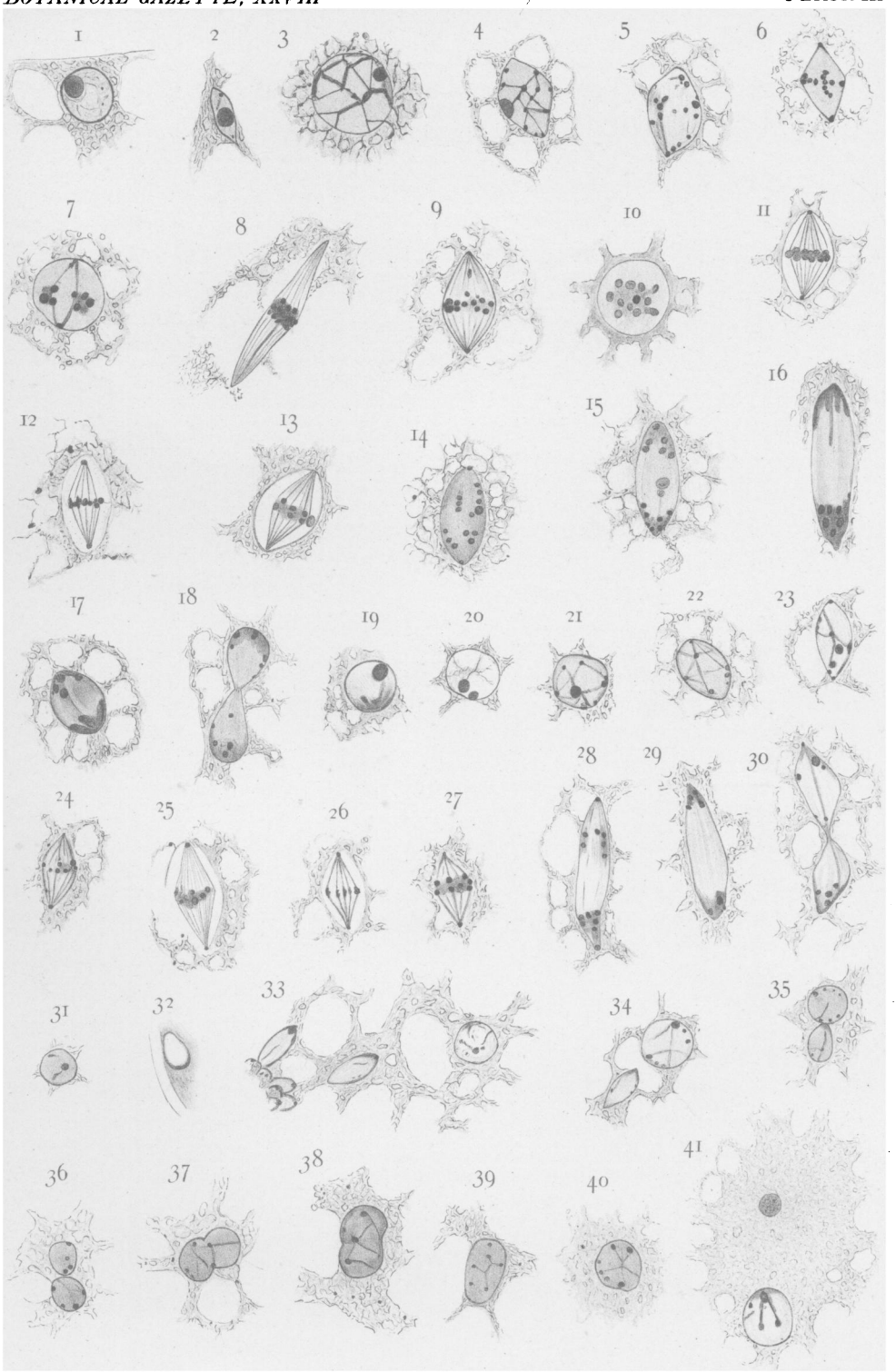
FIG. 81. Diagram of the distribution of oil in an oogonium of the age shown in *fig. 80.*

FIG. 82. Antheridial tube discharging sperms, antheridium shown above, primitive wall very young, ooplasm becoming vacuolate, antheridial tube cut slightly oblique so that its base is in an adjacent section.

FIG. 83. Diagram to show relation of sections presented in *figs. 84, 85, 86: fig. 84* was cut from above the line *aa*, and tangential to the tip of the opening tube; *fig. 85* from between the lines; *fig. 86* from just below the line *bb*.

FIG. 84 (see *fig. 83*). Sperm nuclei leaving the antheridial tube and approaching the female nuclei.

FIG. 85 (see *fig. 83*). Mass of sperms escaping from tube. The tube may be traced to the left as a mass of darkly stained structureless protoplasm.



F.L.S. del. Lith. Anst. v. E. A. Funke, Leipzig.

STEVENS on ALBUGO

The sperms in a mass appear dark but individually are hyaline except at anterior end which bears the nucleolus. See also *fig. 33, Plate XI.*

FIG. 86 (see *fig 83*). The base of the antheridial tube filled with dark staining cytoplasm and few nuclei.

FIG. 87. Just after discharge from antheridial tube, periplasm as in *fig. 82*, several small masses of nuclei apparently both male and female surrounded by denser cytoplasm (these masses seem to arise by the breaking apart of the contents of the antheridial tube and soon disappear), primitive wall well developed, antheridium above, ooplasm coarsely and irregularly vacuolate.

FIG. 88. Nuclei fusing in pairs, primitive wall distinctly thickened. See *Plate XI, figs. 36–40.*

PLATE XV.

All figures magnified 1500 diameters.

FIG. 89. Protoplasm collecting in dense network toward the center of the oospore, leaving light peripheral strands where the endospore is soon to appear.

FIG. 90. Shows the remains of the antheridial tube in the periplasm, with no trace of its former presence in the ooplasm. The oospore is of the age shown in *fig. 88.*

FIG. 91. Primitive wall mature, exospore forming, now consisting of a porous semi-transparent mass with imbedded disks which are to form the ridges, vacuoles in oospore filled with gelatinous substance, nuclei of protoplasm overstained.

FIG. 92. Somewhat younger than the last figure, showing structure of exospore more clearly, presents edge view and also a fragment bent back and giving a surface view, gelatinous substance in vacuoles.

FIG. 93. Exospore nearly formed, primary endospore complete, vacuoles still containing gelatinous substance.

FIG. 94. Rudimentary secondary endospore, gelatinous substance in vacuoles.

FIG. 95. Oil forming in oospore.

FIG. 96. Spore walls complete, including double endospore, vacuoles with no gelatinous substance, oil accumulating in large drops on the endospore walls.

FIG. 97. Winter conditions of exospore, the ridges higher than in previous stages, large central oil-like mass, nuclei in sporoplasm. Section not directly through middle so that the endospore appears thicker than it really is.

FIG. 98. Exterior of ripe oospore.